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ASSESSMENT OF SKIN ERYTHEMA AFTER EXPOSURE TO DIFFERENT DOSES OF METHYL NICOTINATE

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13. ABSTRACT *(Maximum 200 words)*
The purpose of this study was to determine the topical dose of methyl nicotinate (Mnic) that optimized skin blood flow for a 30-minute period. Methyl nicotinate is a topical vasodilator used to evaluate the integrity of skin protection compounds. Specifically, this test dose of Mnic was used to evaluate the ability of SERPACWA (Skin Exposure Reduction Paste Against Chemical Warfare Agents), a recently FDA approved topical skin protectant, to provide an efficacious barrier to this challenge agent. Six subjects were studied in a climate-controlled room (22-24°C, 25-30%rh). Three application sites were marked on the volar surface of each forearm (2.4-cm diameter). A 10 ml sample of each of six aqueous Mnic concentrations (0, 1.25, 2.5, 5, 10, or 25 mMol) was applied to the volar surface of each forearm and removed after two minutes. Laser Doppler Imaging (LDI) was used to measure basal skin blood flow and cutaneous erythema following application of Mnic to each site. The LDI scans were performed prior to Mnic application (baseline) and were repeated approximately every 3 minutes for approximately 30 minutes post Mnic exposure. Statistically, cutaneous erythema was significantly greater with 2.5, 5, 10, and 25 mMol doses compared to 0 and 1.25 mMol doses ($p < 0.05$). Cutaneous erythema was also greater with 5 mMol than with 2.5 mMol ($p < 0.05$). There were no significant differences among the 5, 10 and 25 mMol doses. Cutaneous erythema was greater than baseline at 9 minutes post Mnic exposure ($p < 0.05$) and peaked at 12 minutes post Mnic exposure. Cutaneous erythema was not different between 12 and 25 minutes post Mnic exposure, but was significantly attenuated at 28 minutes compared to 12 minutes post Mnic exposure value ($p < 0.05$). These results indicate that 5 mMol Mnic induces optimal cutaneous erythema between 12 and 25 minutes post Mnic challenge and that increasing the Mnic dose does not further increase cutaneous erythema. Consequently, we used a 5 mMol dose of Mnic to induce cutaneous erythema and measured erythema between 12-25 min after challenge in subsequent efficacy studies of SERPACWA.

14. SUBJECT TERMS
methyl nicotinate (Mnic), vasodilator, SERPACWA, topical skin protectant, cutaneous erythema, laser Doppler imaging,

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EXECUTIVE SUMMARY

The purpose of this study was to determine the topical dose of methyl nicotinate (Mnic) that optimized skin blood flow for a 30-minute period. Methyl nicotinate is a topical vasodilator used to evaluate the integrity of skin protection compounds. Specifically, this test dose of Mnic was used to evaluate the ability of SERPACWA (Skin Exposure Reduction Paste Against Chemical Warfare Agents), a recently FDA approved topical skin protectant, to provide an efficacious barrier to this challenge agent. Six subjects were studied in a climate-controlled room (22-24°C, 25-30%rh). Three application sites were marked on the volar surface of each forearm (2.4-cm diameter). A 10 μl sample of each of six aqueous Mnic concentrations (0, 1.25, 2.5, 5, 10, or 25 mMol) was applied to the volar surface of each forearm and removed after two minutes. Laser Doppler Imaging (LDI) was used to measure basal skin blood flow and cutaneous erythema following application of Mnic to each site. The LDI scans were performed prior to Mnic application (baseline) and were repeated approximately every 3 minutes for approximately 30 minutes post Mnic exposure. Statistically, cutaneous erythema was significantly greater with 2.5, 5, 10, and 25 mMol doses compared to 0 and 1.25 mMol doses ($p<0.05$). Cutaneous erythema was also greater with 5 mMol than with 2.5 mMol ($p<0.05$). There were no significant differences among the 5, 10 and 25 mMol doses. Although cutaneous erythema after the 2.5 mMol doses was significantly greater than 1.25 mMol, the 5 mMol applications produced the least variability in erythemic response among all subjects. Cutaneous erythema was greater than baseline at 9 minutes post Mnic exposure ($p<0.05$) and peaked at 12 minutes post Mnic exposure. Cutaneous erythema was not different between 12 and 25 minutes post Mnic exposure, but was significantly attenuated at 28 minutes compared to 12 minutes post Mnic exposure value ($p<0.05$). These results indicate that 5 mMol Mnic induces optimal cutaneous erythema between 12 and 25 minutes post Mnic challenge and that increasing the Mnic dose does not further increase cutaneous erythema. Consequently, we used a 5 mMol dose of Mnic to induce cutaneous erythema and measured erythema between 12-25 min after challenge in subsequent efficacy studies of SERPACWA.

INTRODUCTION

SCIENTIFIC BACKGROUND

Chemical warfare agents (CWA) continue to be a major threat to U.S. war fighters and peacekeepers. CWA such as the blistering agent, sulfur mustard (HD), and the nerve agents soman (GD), thickened soman (TGD), and VX, increase this threat because of the toxicity and lethality of these agents by percutaneous absorption. The most effective way to protect our soldiers from the effects of these agents is to prevent or limit their exposure.

Chemical protective gear, including the jacket and trouser overgarments, mask, gloves and boots, provide good protection, but the closure sites of this ensemble may be vulnerable to CWA exposure during wear. Topical Skin Protection (TSP), which is now called Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), has been proposed to complement the use of chemical protective clothing as protection against the percutaneous threat of chemical agents at the closure sites of these garments (4). The mechanism of SERPACWA's protection quality is a function of its physicochemical characteristics. SERPACWA is non-reactive, non-wetting and immiscible with nearly all other chemicals. SERPACWA may prevent CWA from making direct contact with the skin.

Studies of the efficacy of SERPACWA have historically utilized a skin challenge with a potential offending agent such as an expected toxin or an allergen. Urushiol (poison ivy) extract has been used in clinical trials to evaluate other topical creams for protection (1) and by the Army to evaluate SERPACWA (1,4). The end point of protection was determined by observing evidence of agent penetration to skin. The observations included erythema and vesiculation some days after the exposure. To date, SERPACWA has been shown to be an effective skin protectant for up to an hour in sweating humans (4). In that study methyl nicotinate (Mnic) was used to evaluate SERPACWA's effectiveness on sweating subjects. The advantage of Mnic as a challenge to a skin protectant is the rapid skin response (non-immunolgic contact reaction) to this agent. The reaction is manifested by a rapid onset of erythema or urtication, generally within minutes of skin exposure to the agent. This response can be quantified by measuring flux (proportional to tissue blood flow) by laser-Doppler Imaging (LDI) techniques. The end point criterion for protection is determined by observing evidence of the non-immunologic contact reaction, such as erythema, and an increase in flux after exposure to the challenge agent, Mnic.

Methyl nicotinate (methyl 3-pyridinecarboxylate), a lipid soluble ester of nicotinic acid produced by Sigma Aldrich Chemicals (St. Louis), is a well-studied contact irritant, which produces easily monitored non-immunologic contact reactions. The non-immunologic contact reaction is due to increased prostaglandin, an inflammatory mediator released after penetration of Mnic through stratum corneum into the dermis (2,3,7,8,12,13,14). Mnic concentrations from 0.1 to 150 mMol produce measurable reactions. In a recent study conducted to evaluate the effectiveness of SERPACWA

under sweating conditions, a 2-minute exposure of 2.5 mMol Mnic resulted in an erythema that was subjectively visible after 2-3 min (4). The erythema increased in intensity between 10 and 20 min post-challenge, and gradually decreased for 20 to 30 min post challenge. This response to an Mnic challenge is observed in a majority of healthy adults.

Application of SERPACWA prior the Mnic challenge has been shown to alter the inflammatory response (4) by limiting or preventing penetration of the agent through the stratum corneum into the dermis. The efficacy of SERPACWA application to sweating subjects was evaluated by measuring the response to an Mnic challenge to SERPACWA treated skin. This response was compared to untreated skin by laser Doppler imaging technology (4).

The new LDI technique (Moor Instruments, England) provides a 2-dimensional pattern of cutaneous microcirculation, which offers a visual image and quantification of the intensity and expansion of perfusion (beyond that which is detectable using standard clinical methods of evaluation). LDI provides a sensitive, accurate, reproducible and noninvasive means of measuring changes in skin blood flow as reported in the scientific literature (5,6,7,8,9,12). The scanning technique is less variable than single point laser technology, and offers the ability to evaluate several test sites simultaneously. The principles of operations of the LDI scan are no different than conventional laser-Doppler scanning technology and a full technical description of the instrument has been published (11).

MILITARY RELEVANCE

Topical Skin Protection (TSP), which is now called Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), has been proposed to complement the use of chemical protective clothing as protection against the percutaneous threat of chemical agents at the closure sites of these garments. The Food and Drug Administration (FDA) recently approved the New Drug Application (NDA #21-084) as a safe and effective topical product for use by soldiers to provide additional protection against CWA. However, the FDA and the Army Combat Developers have requested additional studies of SERPACWA to ascertain conditions that will optimize its effective use by service members, if ever needed during instances of chemical threat.

The U. S. Army Research Institute of Environmental Medicine (USARIEM) agreed to a request from the U. S. Army Medical Materiel Development Activity (USAMMDA) to conduct a clinical study under Good Clinical Practice (GCP) guidelines with the objective of evaluating SERPACWA's effectiveness and durability in protecting the skin from a challenge agent.

PURPOSE

The purpose of this study was to confirm the dose response curve of methyl nicotinate so that the best possible challenge dose could be used to test the duration of effective skin protection by SERPACWA. Based on a previous study (4), it was hypothesized that the asymptote of the cutaneous erythema would be observed approximately 15 min after Mnic application. In effect, we evaluated peak erythema, time to erythema and duration of peak erythema following topical exposure to six different aqueous Mnic doses.

METHODS

TEST SUBJECTS

Six subjects (5 men and 1 woman) between the ages of 18 and 20 volunteered to participate in the study after they were formally briefed on the design and risks of the study. The subjects were enrolled in the study without exclusion for race, ethnicity, or gender. Subjects were nonsmokers; were prohibited from the use of any prescriptive or over-the-counter medications 2 days prior to the experiment; and refrained from alcohol intake 24 hours prior to the experiment. Subjects' volar forearm and wrists were free of any scars, tattoos, or skin disorders such as eczema, psoriasis, or sunburn that would interfere with the erythemic evaluation. Prior to participating, all subjects were medically cleared and tested for a normal erythemic response to a dilute solution of methyl nicotinate (10 μl of a 2.5-mMol Mnic aqueous solution) at a forearm skin site. Responders exhibited a measurable non-immunologic contact reaction to the Mnic challenge as assessed by visual scoring.

ENVIRONMENTAL CONDITIONS

All experiments were performed in a climate controlled room set at normal room temperature (22 to 24°C, 25 to 30% rh).

EXPERIMENTAL DESIGN

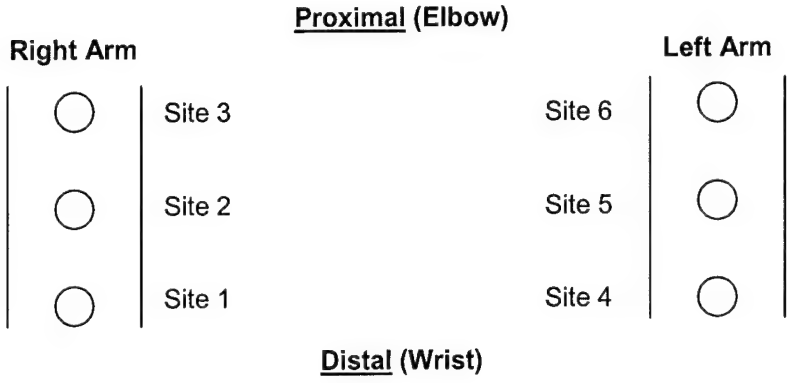
Each volunteer completed a single experiment in which the volar forearm surfaces were exposed to a 10 μl sample of 0, 1.25, 2.5, 5, 10, and 25 mMol of aqueous Mnic.

TEST PROCEDURES

A methyl nicotinate stock solution (50 mMol in distilled water) was prepared from the crystalline solid each test day. All six Mnic challenge solutions were prepared from the stock solution using standard dilution techniques.

A black rectangular template was made for each subject to mark the test area on the volar surface near the wrist of each arm. Three 2.4 cm diameter circular sites separated by 1 cm were identified and marked on the volar surface of each forearm (Figure 1). For each scan, subjects were seated and placed their forearms in a custom made mold that positioned their hands in supination with forearms and wrists close together beneath the LDI unit. The template was repositioned on the forearms prior to initiating the LDI repeat scan mode to provide a contrast for LDI flux graphic display. Subjects were required to wear laser protective goggles during all scans.

Figure 1. Diagram for Mnic Application Sites



The application of each 10 μl Mnic solution (0, 1.25, 2.5, 5, 10, or 25 mMol) was dispensed every 15 seconds beginning with site 1 in a random order by dose using an Eppendorf® Repeater® Pro pipette. After two minutes, the Mnic was removed by using a cotton swab to wick the droplets off each site. The LDI scans were performed prior to Mnic application (baseline) and approximately every 3 minutes after the Mnic was removed for approximately 30 minutes. A total of 9 post-Mnic challenge scans were completed on each of the six subjects. Perfusion units were used to arbitrarily describe erythema. These are known as 'flux': a quantity proportional to the product of the average speed of the blood cells and their number concentration (10).

RESULTS AND DISCUSSION

PEAK CUTANEOUS ERYTHEMA

Using the laser Doppler imaging technique, we were able to quantify the intensity of cutaneous erythema and the duration of cutaneous erythema among the different Mnic concentrations. Table 1 and Figure 3 show that for all Mnic concentrations the cutaneous erythema was greater with 2.5, 5, 10, and 25 mMol Mnic doses compared to 0 and 1.25 mMol doses ($p<0.05$). There was also a significant difference between the 2.5 and 5.0 mMol solutions. The flux values were not different among the 5.0, 10.0, and 25.0 mMol (Figure 3). The mean flux values of each subject calculated for each Mnic dose are graphed in Figure 2. On visual inspection of the individual data, it appeared that the 2.5 mMol dose provided adequate cutaneous erythema response among the individual subjects. However, the mean flux values plotted in Figure 3 indicated a "break point" between doses 2.5 and 5.0 mMol solutions.

Figure 2. Flux Response per Subject for each Mnic dose

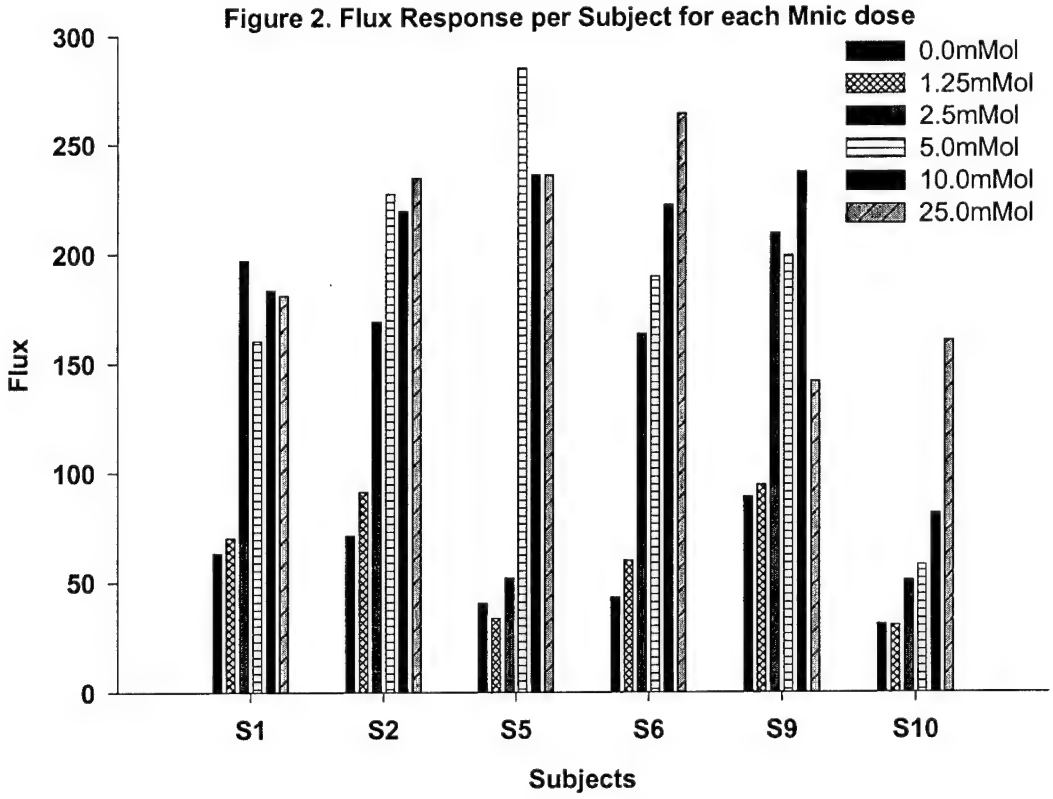
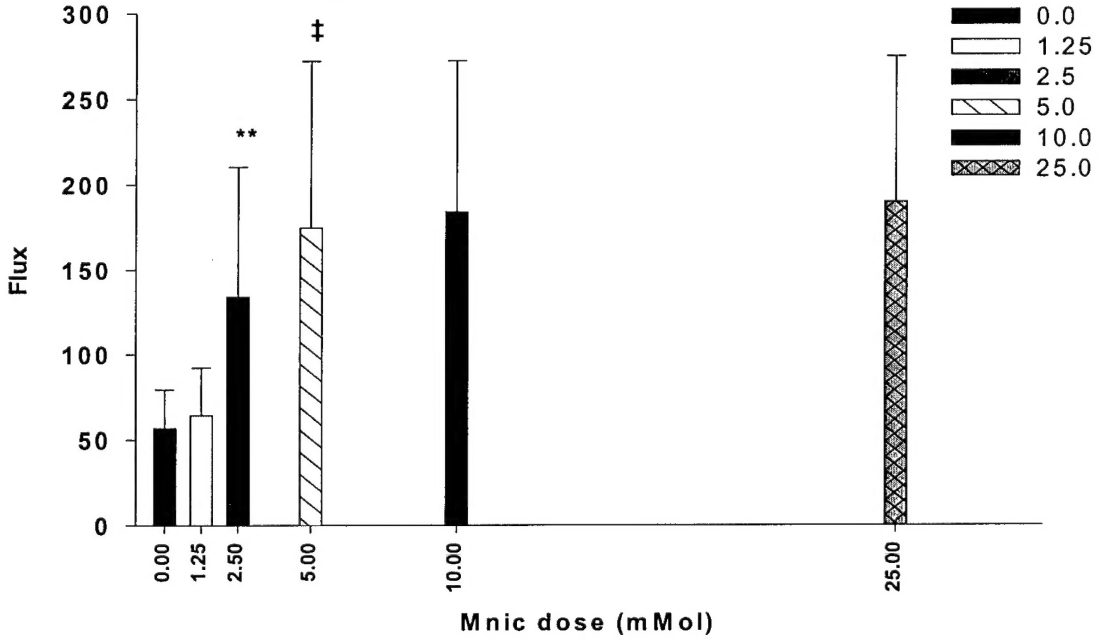


Table 1. Summary flux values for the Mnic (mMol) topical doses

	0.0	1.25	2.5	5.0	10.0	25.0
S01	63.29	70.32	196.91	160.33	183.43	181.06
S02	71.22	91.22	169.06	227.24	219.42	234.38
S05	40.38	33.68	51.84	284.90	235.91	235.84
S06	43.01	59.86	163.46	189.53	222.18	264.00
S09	88.99	94.28	209.29	199.13	237.03	141.66
S10	30.91	30.39	50.74	57.81	81.24	160.26
Mean	**56.30**	**63.29**	**140.22****	**186.49**‡**	**196.54**‡**	**202.87**‡**
SD	21.98	27.43	70.95	75.84	59.73	48.69

Data were compiled from 9 post-Mnic scans. Mean flux values of each subject averaged for each dose. **P<0.05 vs. 0.0 and 1.25 mMol, ‡P<0.05 vs. 2.5 mMol.

Figure 3. Mean Flux Response per Dose



PEAK ERYTHEMA DURATION

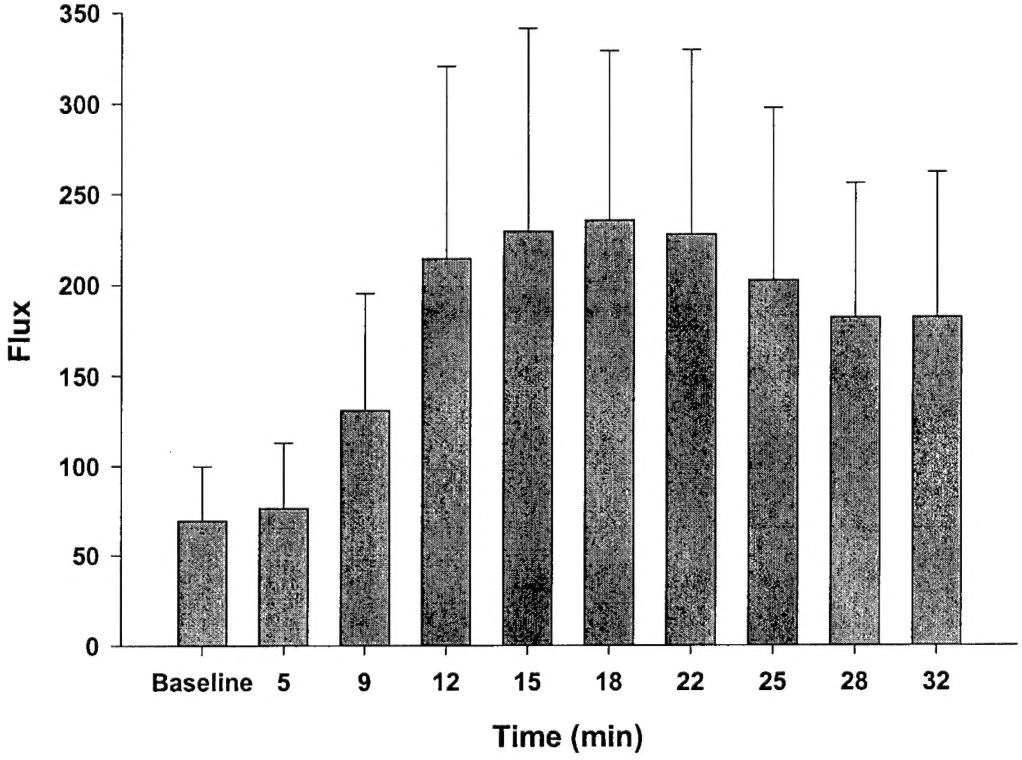
There was no significant difference between baseline and 5 minutes post 5 mMol Mnic challenge in cutaneous erythema. These data are shown in Table 2. However, cutaneous erythema was greater than baseline at 9 minutes post Mnic exposure and reached a plateau at 12 minutes post Mnic exposure. Figure 4 shows the mean and standard deviation of cutaneous erythema over time. Cutaneous erythema was not different from 12 to 25 minutes post Mnic exposure, but was lower at 28 minutes compared to 12 minutes post Mnic exposure value ($p<0.05$).

Table 2. Change in flux measured by LDI imaging

	Baseline	5 min	9 min	12 min	15 min	18 min	22 min	25 min	28 min	32 min
S01	65.10	104.50	140.20	189.00	184.10	175.30	162.20	180.00	153.90	153.80
S02	87.20	79.10	117.00	273.60	298.30	286.40	266.90	219.20	245.80	258.90
S05	52.60	47.90	105.70	351.30	381.60	392.50	383.60	349.50	276.70	275.30
S06	48.60	77.00	199.90	221.30	243.50	225.90	239.30	177.70	162.90	158.30
S09	121.60	124.70	195.40	217.60	217.70	203.80	231.30	230.40	183.00	188.30
S10	40.60	24.30	27.10	31.50	50.00	127.10	81.30	56.20	66.80	56.00
Mean	**69.28**	**76.25**	**130.88****	**214.05**†**	**229.20****	**235.17****	**227.43****	**202.17****	**181.52**‡**	**181.77****
SD	**30.35**	**36.49**	**64.20**	**106.21**	**111.79**	**93.50**	**101.70**	**95.07**	**74.11**	**79.86**

Data were compiled from flux values measured within the 5 mMol site only. **P<0.05 vs. baseline and 5 min, †P<0.05 vs. 9 min, ‡P<0.05 vs. 12 min.

Figure 4. Duration of Peak Blood Flow (5mMol)



CONCLUSIONS

From these data we concluded that laser Doppler imaging technology is advantageous for assessing cutaneous erythema after drug treatment. The application of 5.0 mMol Mnic induced optimal cutaneous erythema so that dose should be used in the evaluation of a topical skin protectant compound. Based on the repeated image scans following the different doses of challenge agent, we concluded that 12 minutes post Mnic challenge would provide adequate time to reach peak flow. Therefore, the 5.0 mMol dose of Mnic and the time window for peak cutaneous erythema was recommended for use in future studies evaluating SERPACWA's effectiveness and durability.

REFERENCES

1. Anonymous. Investigator's Brochure; Topical Skin Protectant ICD 2289. Dec 15, 2000. U.S. Army Medical Research and Material Command, Fort Detrick, MD.
2. Basketter, D. A., and K. P. Wilhem. Studies on non-immune immediate contact reactions in an unselected population. *Contact Dermatitis*. 35: 237-240, 1996.
3. Berardesca, E., M. Cespa, N. Farinelli, G. Rabbiosi, and H. I. Maibach. In vivo transcutaneous penetration of nicotinates and sensitive skin. *Contact Dermatitis*. 25: 1-28, 1993.
4. Cunningham, W., and L. M. Harnisch. *The protective efficacy of the Topical Skin Protectant ("TSP") against methyl nicotinate under sweating conditions*. Fort Detrick, MD: USAMMD. HSRRB # A-8522, June 1999.
5. Duteil, L., C. Queille, M. Poncet, and J. Czernielewski. Processing and statistical analysis of laser Doppler data applied to the assessment of systemic anti-inflammatory drugs. *J Derm. Sci*. 2: 376-382, 1991.
6. Eslner, P., and H. I. Maibach. Cutaneous responses to topical methyl nicotinate in human forearm and vulvar skin. *J Derm. Sci*. 2: 341-345, 1991.
7. Issachar, N., Y. Gall, M. T. Borrel, and M. C. Poelman. Noninvasive assessments of percutaneous absorption of methyl nicotinate in humans. *J Pharm. Sci*. 72: 1077-1079, 1983.
8. Kompaore, F., and H. Tsurata. In vivo differences between Asian, black and white in the stratum corneum barrier function. *Int. Arch. Occ. Environ. Health*. 65: S223-S225, 1993.
9. Lahti, A., H. Kopola, A. Harila, R. Myllyla, and M. Hannuksela. Assessment of skin erythema by eye, laser Doppler flowmeter, spectroradiometer, two-channel erythema meter and Minolta chroma meter. *Arch. Derm. Res*. 285: 278-282, 1993.
10. *moorLDI User Guide and Reference*. Version 3.0. Devon, England: Moor Instruments Limited, 1996.
11. Quinn, A. G., J. Mclelland, T. Essex, and P. M. Farr. Measurement of cutaneous inflammatory reactions using a scanning laser-Doppler velocimeter. *Br. J. Derm*. 125: 30-37, 1991.
12. Reinberg, A. E., E. Soudant, C. Koulbanis, R. Bazin, A. Nicolai, M. Mechkouri, and Y. Touitou. Circadian dosing time dependency in the forearm skin penetration of methyl and hexyl nicotinate. *Life Sci*. 57 1507-1513, 1995.
13. Tur, E., H. I. Maibach, and R. H. Guy. Percutaneous penetration methyl nicotinate at three anatomic sites: evidence for an appendageal contribution to transport? *Skin Pharm*. 4: 230-234, 1991.
14. Wilkin, J. K., G. Fortner, L. A. Reinhardt, O. V. Flowers, S. J. Kilpatrick, and W. C. Streeter. Prostaglandins and nicotinate-provoked increase in cutaneous blood flow. *Clin. Pharm. Therap*. 38: 273-277, 1985.